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Towards personalized medicine with a three-dimensional micro-scale perfusion-based two-chamber tissue model system

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Abstract

A three-dimensional micro-scale perfusion-based two-chamber (3D- μ PTC) tissue model system was developed to test the cytotoxicity of anticancer drugs in conjunction with liver metabolism. Liver cells with different cytochrome P450 (CYP) subtypes and glioblastoma multiforme (GBM) brain cancer cells were cultured in two separate chambers connected in tandem. Both chambers contained a 3D tissue engineering scaffold fabricated with biodegradable poly(lactic acid) (PLA) using a solvent-free approach. We used this model system to test the cytotoxicity of anticancer drugs, including temozolomide (TMZ) and ifosfamide (IFO). With the liver cells, TMZ showed a much lower toxicity to GBM cells under both 2D and 3D cell culture conditions. Comparing 2D, GBM cells cultured in 3D had much high viability under TMZ treatment. IFO was used to test the CYP-related metabolic effects. Cells with different expression levels of CYP3A4 differed dramatically in their ability to activate IFO, which led to strong metabolism-dependent cytotoxicity to GBM cells. These results demonstrate that our 3D- μ PTC system could provide a more physiologically realistic *in vitro* environment than the current 2D monolayers for testing metabolism-dependent toxicity of anticancer drugs. It could therefore be used as an important platform for better prediction of drug dosing and schedule towards personalized medicine.

Keywords

Glioblastoma; 3D cell culture; Micro-scale tissue model system; Two-chamber system; Liver CYP metabolism; Metabolism-dependent toxicity

1. Introduction

Personalized medicine is a new healthcare paradigm where proper medication and dosage are customized to special characteristics of a patient and his/her disease. Individuals respond differently to drugs. A growing list of genetic polymorphisms in drug-metabolizing enzymes, drug transporters and drug targets have been linked to the efficacy, dosage, and toxicity profile in human [1]. Among drug-metabolizing enzymes, the cytochrome P450

(CYP) superfamily converts drugs into their primary metabolites. About 75% of drugs are eliminated via CYP-mediated metabolism, and specifically, CYP3A4, the most abundantly expressed hepatic cytochrome P450, is involved in the metabolic process of two-thirds of all marketed drugs [2]. The genotypes of major drug-metabolizing CYPs vary significantly from person to person. This variability is believed to have resulted in different isozyme activities and contributed to the variation in drug responses among individuals [3]. Differentiating the effect of liver metabolism on drugs is therefore an important aspect of personalized medicine.

Many *in vitro* liver models have been developed to mimic the human liver function in the past few decades. Early liver models included isolated and perfused livers [4,5], liver tissue slices [6-9], freshly isolated hepatocytes in suspension [10,11], primary hepatocyte cultures [12-14], and liver microsomes [15,16]. Each of these models could reproduce certain liver functions; however, the preservation of major liver functions such as drug metabolism tended to be short-lived and the ability to integrate them into a high throughput drug screening system was lacking [17,18]. For high throughput applications, Griffith's group proposed a perfusion-based cell culture system with liver cells cultured in an array of silicon micro-wells [18-21]. Liver cells were successfully cultured for 14 days without a loss of their major functionality. Zhang *et al.* developed a sandwiched platform with primary rat hepatocytes cultured on a collagen-coated polyethylene terephthalate (PET) film [22]. This film was then covered with either polycarbonate or Si₃N₄ porous films and transferred to a 96-well compatible holder with multiple wells connected in series. Chang *et al.* demonstrated an *in vitro* liver model with HepG2 hepatoma cells encapsulated in hydrogel using a syringe-based bioprinting process [23,24]. The functionality of the hepatic cells was shown by administering a prodrug through the model and observing the fluorescence change of the outgoing flow. All the above systems focused on hepatic cells only and no other cells were involved. Shuler's group on the other hand developed a micro-cell culture analog (μ CCA) device, which could be used to study the multi-organ interaction on drug toxicity [25]. The core component of the original μ CCA device is a silicon chip with micro-chambers and connecting channels fabricated with deep reactive ion etching (DRIE). Different types of cells including liver, cancer and bone marrow cells were cultured in these chambers. However, this silicon-based device caused cells to attach to the bottoms of the micro-chambers and form monolayers instead of the desired three-dimensional (3D) construct.

When it comes to cancer drug related research, 3D tissue constructs are highly desirable, since they better mimic *in vivo* structures of cancerous tissues. It is well established that *in vivo* cancer progression is modulated by the host and the tumor micro-environments, which are mostly 3D [26,27]. There is increasing evidence that cells growing in 3D are more resistant to cytotoxic agents than those in monolayers [28,29]. In addition, 3D cell cultures could foster new insights into tumorigenesis [30] and they show significantly increased hematopoietic differentiation efficacy for embryonic stem cells [31]. There have been several efforts developing 3D tissue constructs for drug screening applications. For example, some have stacked up multiple microfluidic layers, each with patterned wells and channels [32]; others encapsulated cells in hydrogel and allowed the perfusion media to flow by the side of the construct [33,34]. The limitations of these systems include the fabrication challenges and inadequate diffusion of nutrients or drugs into thick hydrogel constructs [35].

In this paper, we report a 3D micro-scale perfusion-based two-chamber (3D- μ PTC) tissue model system for cancer drug testing. The system is built on a polymer chip with two separate chambers containing porous polymeric scaffolds for liver and cancer cell cultures, respectively. We used the system to test the cytotoxicity of anticancer drugs, including the liver metabolism effects with varying CYP3A4 expression levels. The expression of CYP

enzymes can change due to promoter and enhancer polymorphisms, sex, age, and environmental exposures [3,36-38]. The ability to differentiate the effects of different CYP activity levels will have strong implications in personalized medicine.

2. Materials and methods

2.1. Cells and chemicals

Aggressive brain tumor cells representing glioblastoma multiforme (GBM) (cell line M059K) were obtained from American Type Culture Collection (ATCC, Manassas, VA). Three cell types of hepatic origin were tested. The hepatoblastoma HepG2 and its derivative C3A cell lines were purchased from ATCC. The C3A-sub28 cell line with enhanced expression of CYP3A4 mRNA and CYP3A4-mediated activity was generated from C3A cells at the University of Eastern Finland [39]. All the cells were maintained in 25 cm² T-flasks with Dulbecco's Modified Eagle Medium/Nutrient Mixture F-12 (DMEM/F12) (1:1) cell culture medium and 10% fetal bovine serum (FBS) (Invitrogen, Carlsbad, CA). The T-flasks were placed in an incubator (Thermo, Boston, MA) maintained at 37 °C and 5% CO₂.

Cancer treatment drugs, temozolomide (TMZ) and ifosfamide (IFO), were obtained from Sigma Aldrich (St Louis, MO). The chemical structures of TMZ and IFO are shown in Fig. 1. TMZ is an anticancer drug widely used for the treatment of GBM. It does not require metabolic activation by hepatic CYPs [40]. IFO, on the other hand, is a prodrug that requires CYP-dependent activation for its antitumor activity.

Live/dead stain assay and Alamar blue assay were used to evaluate the cell viability. The live/dead staining solution was obtained from Invitrogen Inc., CA. The Alamar blue assay kit was acquired from Fisher Scientific Inc., Pittsburgh, PA.

2.2. Device design and fabrication

The 3D tissue model device was fabricated with polycarbonate (PC) (Makrolon, USA). The device had a center piece and a top and a bottom cover, as shown in Fig. 2. The diameter of the cylindrical chambers in the center piece was 13 mm and the depth was 2.5 mm. Porous scaffolds were placed in the two chambers with cell retaining membranes (0.8 μm pore size from Millipore) attached to the bottoms. Recess shoulders were machined to set the scaffolds in place. Fluid channels were machined to connect the two chambers. Two 25G stainless steel needles were embedded in the device as inlet and outlet of the fluid system. All the machining was done on a CNC mill. The bottom cover was attached to the center body with biomedical-grade super glue (Sigma Aldrich, USA). The top cover was assembled with stainless steel screws. In between the device body and top cover, a gasket made of polydimethylsiloxane (PDMS from Dow Corning, USA) was used to prevent possible leaks.

The porous scaffolds used in the tissue model device were prepared in house using a solvent-free fabrication approach [41,42]. The scaffold material was biodegradable polylactic acid (WMI, Taiwan). The fabrication process consisted of two steps. In the first step, PLA samples were foamed in a solid-state foaming process, where the pore size was controlled with the foaming process parameters. In the second step, foamed PLA samples were treated with 20 kHz power ultrasound to break the pore walls in the PLA foam. Fabricated PLA scaffolds were then cut into disc-shaped samples. Fig. 3 shows the shape and microstructure of the fabricated scaffolds. The diameter of the samples was 13 mm and the thickness was 2 mm. The average pore size was 300 μm and the average porosity was 70%.

The two-chamber tissue model device was connected in a medium circulation system driven by a peristaltic pump (ISMATEC, ISM 936D). Fig. 4 shows the entire system maintained in an incubator for cell culture and drug testing. A 25 ml glass vial was used as a reservoir for the cell culture medium. For drug studies, controlled doses of anticancer drugs were added into the medium reservoir for circulation through the liver and cancer chambers. Experiments were conducted to determine the appropriate flow rate for the media perfusion.

2.3. Sterilization and cell culture

Before assembling the circulation system, components of the 3D tissue model device including the polycarbonate membranes, stainless steel screws, connecting tubing (0.38 mm inner diameter), and the glass vial were all sterilized in an autoclave at 122 °C for 1 h. The PLA scaffolds needed separate sterilization before cell seeding. They were rinsed with distilled water several times and then soaked in a 1% bleach solution and subsequently in 70% ethanol for 30 min each. Dried scaffolds were exposed under ultraviolet in the bio-safety hood for additional 30 min. After that the scaffolds were immersed into a complete cell culture medium (DMEM/F12 1:1) containing 10% FBS and incubated for three days before cell seeding.

For 3D cell cultures, two PLA scaffolds were loaded into the liver and cancer chambers. Approximately 5×10^4 liver cells and GBM cancer cells were seeded with 200 μ l culture medium separately onto the two scaffolds. For a control study, only GBM cancer cells were seeded. The liver chamber was left empty. For 2D cell cultures, the cells were seeded directly on the PC membrane without the PLA scaffolds. The seeded tissue model device was incubated at 37 °C with 5% CO₂ for 24 h. This allowed cell attachment to the scaffolds or the PC membranes in the 2D cell culture case. The whole tissue model system was then assembled by attaching the top cover, connecting tubing, and the peristaltic pump.

2.4. Cell viability assessment

To measure the cell viability, the 3D PLA scaffolds (or the PC membranes in 2D cultures) were transferred into a clean 24-well plate. 450 μ l culture medium was added with 50 μ l Alamar blue dye into each well. The plate was incubated for additional 2 h, and then scanned with an IsoCyte laser scanning cytometer (Blueshift Biotechnologies Inc., Sunnyvale, CA). The laser scanning cytometer uses a laser source to excite the fluorescence signal point-by-point and detect the emission signals with highly-sensitive photomultiplier tubes. The fluorescence signal was detected at 580–610 nm emission wavelength band with the 540–570 nm excitation band. The fluorescence intensity was correlated to the number of viable cells in the wells. Cell viability inside the PLA scaffolds was also examined using Live/Dead staining and Haematoxylin and Eosin (H&E) staining. A fluorescent stereomicroscope (LEICA M205FA) was used to obtain Live/Dead images over time. Samples for H&E staining were prepared after four days of perfusion cell culture and examined with an optical microscope.

All data are expressed as mean $\pm$ standard deviation (SD). The Student *t*-test was used to analyze the statistical significance of data pairs. While dealing with multiple groups of data, one-way ANOVA test was used. The significance was considered when $p < 0.05$. A p -value larger than 0.05 ($p > 0.05$) was taken as indication of no significant difference.

3. Test results

3.1. Flow rate effects on cell viability

Flow-induced shear stress strongly affects the cell adhesion, proliferation, and viability in a perfusion-based cell culture system [43-46]. Therefore, one of the critical steps in this study

was to determine a suitable flow rate for the perfusion system. We have tested the system with three perfusion rates: 10, 5, and 2.15 $\mu\text{l}/\text{min}$. Fig. 5 shows the cell viability under the different perfusion flow rates after 24 h of culture. The PLA scaffolds were seeded with 5×10^4 liver and GBM cancer cells each in the beginning. It can be seen that a lower flow rate led to much higher cell viability. In the HepG2 chamber, the cell viability increased by more than four times when the flow rate dropped from 10 to 2.15 $\mu\text{l}/\text{min}$. In the GBM chamber, the cell viability increased by more than two times for the same flow rate reduction. It is known that a higher flow rate would cause a higher shear stress, which in turn would hamper cell attachment or cause more cell damage. Moreover, a higher flow rate would leave less time for diffusion in the porous scaffold, which would cause insufficient nutrition uptake by the cells. In this study, we used 2.15 $\mu\text{l}/\text{min}$ as the perfusion flow rate in the rest of the tests.

3.2. Cell culture results

After seeding the cells onto the PLA scaffolds, an additional hour was allowed for cell attachment. The majority of the cells were retained in the 3D scaffolds. However, it was possible that a small number of cells did not attach. The cell retention efficiency was thus determined by removing the PLA scaffold and measuring the number of cells on the PC membrane. Based on the results from ten separate seeding tests, the cell retention efficiency were determined to be above 80% for both HepG2 and GBM cells.

Fig. 6 shows the cell culture results of HepG2 and GBM cells after 96 h of perfusion. As a comparison, cell cultures in both 3D and 2D were conducted. It is seen that the cells maintained high viability in both cases. However, in the 2D culture, the cells tended to spread out to form a monolayer, as seen in Fig. 6(a) and (b). When cultured in 3D, these cells aggregated and formed organoid structures (Fig. 6(c) and (d)). To confirm these organoid structures inside the 3D PLA scaffolds, histological H & E staining was performed with HepG2 cell culture samples. As shown in Fig. 7, the aggregated spheroids were clearly observed in the pores of the scaffolds.

Currently, cancer drug screening is mostly done using monolayer cell cultures in Petri dishes and microtiter plates. The intrinsic drawback of these 2D systems is that they cannot reproduce the dynamic 3D microenvironment of the extra cellular matrix (ECM) *in vivo*. Instead, cells are forced into an artificial monolayer and lose certain functions found in native 3D tissues [47,48]. The cell culture experiments in this study have shown that 3D organoid tissue structures could be achieved in our PLA scaffold-based tissue model device. It is generally thought that these tissue structures can better mimic the *in vivo* cellular response [49,50].

3.3. Effects of 3D cell culture and liver metabolism

To demonstrate the effects of 3D cell culture and liver metabolism, the cytotoxicity of TMZ to GBM cells was tested using the 2D and 3D cell cultures with and without the HepG2 hepatic cells. Two types of experiments including both time- and dose-dependent drug response assays were conducted. Fig. 9 shows the results from the time-dependent drug assay. Cell viability was calculated as the ratio of cell numbers in the test sample to that in the control sample, where no drug is applied. Experiments were repeated at least twice and at least three measurements were taken for each experiment. For all the tests in the time-dependant assays, 100 μM drug was added to the medium reservoir and circulated through the system. Cell viability after 24, 48, 72, and 96 h were assessed with the fluorescence intensity measurement.

As seen in Fig. 8, while in general the number of viable cells decreased over time, there was a significant difference between the 2D and 3D culture conditions ($p < 0.05$). On average,

the GBM cell viability in the 3D culture was almost three times higher than in the 2D monolayer culture. This result was true regardless of the presence of hepatic cells. With the HepG2 cells, the viability of GBM cells in 3D was 1.7–3.8 times higher than that in 2D over a 1–4 day time period. Without the liver cells, it was 2.3–3.3 times. This observation indicated that cells cultured in 3D had much higher drug resistance than those in 2D monolayers.

Fig. 8 also shows that there was significant difference in the GBM cell viability depending on the presence of HepG2 cells in the system. TMZ showed a significantly lowered toxicity to the GBM cancer cells when HepG2 cells were present, irrespective of the 2D or 3D culturing conditions. On average the GBM cancer cell viability was about 70% higher over the 1–4 day time period when the liver cells present.

The results from the dose-dependent drug assays are shown in Fig. 9. A gradient of TMZ concentrations of 100, 50, 20, and 10 μM were injected into the system 24 h after cell seeding. Cell viability was measured after another 24 h. Each experiment was repeated twice and at least three measurements were taken at each data point. Four cell viability dose-response curves were constructed, each representing either a 2D or 3D culture and with or without HepG2 cells. While all curves showed a similar trend of increased cytotoxicity to GBM cells upon increased TMZ concentrations, there were again significant differences among the four cases. It was clear that the cells in 3D were much more drug resistant than in 2D, which was consistent with the observation in [51], and that the presence of hepatic cells reduced the cytotoxicity of TMZ in both culture formats. In the monolayer culture, we found that the half-maximal inhibitory concentration (IC_{50}) for TMZ was about 8 μM in the absence of HepG2 cells, similar to a previously reported IC_{50} of 11 μM [52]. However, when the drug was passed through the liver cells, the IC_{50} rose to about 18 μM .

The dependence of TMZ toxicity on the presence of hepatic cells can be explained with its metabolic pathway. The therapeutic effect of TMZ is based on its hydrolytic product 5-(3-methyltriazene-1-yl)-imidazo-4-carboximide (MTIC), which has an ability to alkylate DNA and trigger the death of GBM cancer cells [53]. The conversion of TMZ to MTIC is a spontaneous transformation under physiological conditions [40]. However, MTIC can break down irreversibly to a non-cytotoxic compound 5(4)-aminoimidazole-4(5)-carboxamide (AIC) in liver cells at an acid pH level [54]. In aqueous buffers, TMZ is stable at pH <4, but rapidly decomposes to cytotoxic MTIC at pH >7. Conversely, MTIC is stable at pH >7, but breaks down to non-cytotoxic AIC after being metabolized by hepatic cells at pH <7. Fig. 10 depicts this metabolic pathway of TMZ. Thus, the biotransformation of cytotoxic MTIC into non-cytotoxic AIC by HepG2 cells is the most plausible reason behind the lower cytotoxicity of TMZ to GBM cancer cells seen in Figs. 8 and 9. As most chemotherapy drugs undergo first-pass liver metabolism by the liver before reaching cancerous tissues, our results suggest that the effects of liver metabolism should be considered when determining drug doses and schedules for cancer treatment.

3.4. Effects of CYP3A4 expression levels

The effect of liver metabolism was also tested by assessing the cytotoxicity of ifosfamide (IFO) to GBM cells. IFO is a nitrogen mustard alkylating agent used for various tumors treatment for decades. By itself, IFO is only slightly cytotoxic and needs to be activated by CYP enzymes. As shown in Fig. 11, the bioactivation and metabolism of IFO is largely dependent on CYP3A4 [55]. Among various IFO metabolites, isophosphoramidate mustard (IPM) is the most important compound that has the capability of causing irreparable cross-link between DNA double strands, thus blocking the DNA replication and leading to the death of cancer cells [56].

To simulate the variability of liver metabolism among individual patients, cell lines C3A and C3A-sub28 were used. The C3A line is a clonal derivative of HepG2 and it has a strong contact inhibition of growth, high albumin and alpha-fetoprotein (AFP) production, and the ability to grow in glucose-deficient media. C3A-sub28 is a derivative of C3A cell line which displays significant increases in CYP3A4 (15-fold), CYP2B6 (18-fold) and CYP2C9 (142-fold) mRNA expression and about 8-fold increase in CYP3A4-mediated metabolic activity as compared to the wild-type C3A cells [39].

Again, both time- and dose-dependent responses were tested. In the time-dependent response assay, 1000 μM IFO was added to the culture medium and the cell viability was measured every 24 h for three days. Fig. 12 shows the time-dependent response of IFO with C3A and C3A-sub28 liver cells, as compared to the situation where IFO was given directly to GBM cells. In contrast to TMZ, the viability of GBM cells was much lower in the presence of hepatic cells. Without hepatic cells, IFO had only a slight toxic effect. In addition, the C3A-sub28 cell line displayed a much stronger effect in reducing the GBM cell viability than the parent C3A cells. The C3A-sub28 line contains a higher level of CYP3A4, which is able to convert IFO into cytotoxic IPM; therefore, increased CYP3A4 levels will result in increased rates of IPM formation and consequently, a lower cell viability of the GBM cells. These observations confirm that IFO must be bioactivated by hepatic cells. More importantly, they indicate that the variability of liver metabolism can cause differences in effectiveness of cancer drugs.

Fig. 13 shows the results from the dose-response study. Different concentrations of IFO ranging from 100 to 1000 μM were added to the culture medium separately and the viability of GBM cells was measured after 24 h of exposure to the drug. It is seen that the cell viability generally decreased with the increase of drug concentration. In the absence of hepatic cells, GBM cells showed a much higher viability ($p < 0.01$). Moreover, GBM cells showed a significantly higher viability in the presence of C3A cells than that of C3A-sub28 cells ($p < 0.05$).

4. Discussion

There are 57 active human CYP genes of which about 10 are involved in drug metabolism [57]. Genetic polymorphisms in CYP genes are common and may cause loss of enzyme activities, diminished or increased enzyme expression levels, and enzymes with altered substrate specificities. Also, exogenous factors tend to either induce or repress CYP expression levels [58] resulting in a major impact on the pharmacokinetics and metabolism of most drugs. Our study with hepatic cell lines indicates that different CYP3A4 expression levels could have a substantial impact on the effects of chemotherapeutic drugs. Therefore, consideration of CYP genotypes and expression levels for determination of drug doses and schedules could bring tremendous benefits to personalized treatment of diseases. The 3D tissue model device developed in this study could provide an important platform to investigate and predict drug effectiveness among individual patients.

Our two-chamber device utilizes porous PLA scaffolds to engineer 3D tissue models under perfusion conditions. The 3D porous scaffolds provide an *in vivo*-like cellular microenvironment that promotes cell–cell and cell–matrix interactions. Cells cultured in such a 3D environment are likely to form complex structures that preserve cellular functions important *in vivo*. It is well known that cells in 3D respond better to microenvironmental cues and chemical or mechanical signals than those in 2D cell cultures for cell differentiation, proliferation, metabolism, and metastasis [46,51,59,60]. Also, cellular response to chemotherapeutic agents can be drastically different between 2D and 3D cultures, varying as much as 1000 folds [61]. In contrast to monolayers, the cells cultured in

3D are tightly packed to form a multilayer structure, which impedes the diffusion of drug molecules thus providing physical protection to the cells inside. In addition, cells cultured in 3D could alter their gene expression that would make them more resistant to anticancer drugs [62]. These drug resistance effects have not been seen in 2D cell cultures and can only be faithfully represented using a 3D tissue model system.

Our 3D porous scaffolds also provide a better mass transfer environment for cell culture and drug testing. In porous media, diffusion is the dominant process for mass transfer. Tissue formation could fail because of inadequate diffusion of nutrients [35]. Many existing 3D cell cultures were conducted in hydrogels and the cells often grew at a slower pace and remained scattered due to the diffusion limitation. In gel-based dual cell culture models, the fluid flow was arranged from the side or from the top of the system. The nutrient and drug molecules must diffuse through the hydrogel to reach the cells, and hepatic cell-derived metabolites need to diffuse into the flow and then reach the cancer cells. This method may cause insufficient stimulation to the cells. Compared to hydrogels, the diffusion of nutrients in our porous scaffolds is much faster due to the relatively large pores. The higher diffusivity in porous scaffolds could facilitate better cell growth and the reformulation of tissue-specific structures [63-68]. Moreover, our 3D tissue model device allows a more direct contact between the medium and the hepatic and cancer cell aggregates as it flows through the entire system, as shown in Fig. 14. Such direct contacts ensure that the drug is sufficiently metabolized by the hepatic cells and the drug metabolites sufficiently utilized by the cancer cells, as they are carried through the system by perfusion.

5. Conclusions

A three-dimensional micro-scale perfusion-based two-chamber (3D- μ PTC) tissue model system was developed to test the cytotoxicity of anticancer drugs to glioblastoma multiforme (GBM) cells. The effects of hepatic metabolism were tested with cell lines of different CYP3A4 expression levels. The 3D porous scaffolds used in this study promoted the formation of *in vivo* tissue-like organoids and ensured better cell-medium interaction due to the large pores of the scaffolds. Cells cultured in 3D showed a much higher resistance to anticancer drugs than those in 2D. The effects of metabolism depended on the type of anticancer drugs. Hepatic metabolism reduced the effectiveness of temozolomide while the prodrug ifosfamide required hepatic metabolism to induce GBM cytotoxicity. The developed 3D- μ PTC device allowed us to study the effects of different CYP expression levels on cancer drug toxicity. Hepatic cells over-expressing CYP3A4 produced significantly higher cytotoxicity to GBM cells when exposed to ifosfamide. These results demonstrate that our 3D two-chamber system can provide a more physiologically realistic *in vitro* environment than the current 2D monolayers for testing metabolism-dependent toxicity of anticancer drugs in various settings. It could provide an important platform for anticancer drug therapy by contributing towards better prediction of drug dosing and schedule in personalized medicine.

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Fig. 1.
Chemical structures of TMZ and IFO.

Fig. 2.
(a) An assembled 3D tissue model device. (b) Components of the 3D tissue model device.

Fig. 3.
(a) Disc-shaped PLA scaffolds, scale bar 10 mm; and (b) scanning electron microscope image of the cross section of a PLA scaffold, scale bar 500 μm .

Fig. 4.
The 3D tissue model system: (a) a schematic, and (b) the setup.

Fig. 5. Experimental results of cell viability under different flow rates after 24 h of perfusion culture. The 3D scaffolds were seeded with 5×10^4 HepG2 and GBM cancer cells, respectively. The error bars show mean $\pm$ SD of three independent replicates, *: $p < 0.05$.

Fig. 6. Fluorescent images of both HepG2 and GBM cells after 96 h of perfusion cell culture. (a) HepG2 cells on a PC membrane; (b) GBM cells on a PC membrane; (c) HepG2 cells inside a PLA scaffold; (d) GBM cells inside a PLA scaffold. The scale bars are all 200 μm .

Fig. 7.
An H&E staining image of the HepG2 cells inside a PLA scaffold after 96 h of perfusion cell culture. The scale bar is 100 μm .

Fig. 8. Comparison of GBM cancer cell viability in time-dependent tests. Cells were exposed to 100 μ M TMZ and cultured for 24, 48, 72, 96 h. The results were presented as mean $\pm$ SD of two independent replicates.

Fig. 9. Results from the dosage-dependent assays. Cells were exposed to 0, 10, 20, 50, 100 μM TMZ and cultured for 24 h.

Fig. 10. Chemical structures of TMZ, 5-(3-methyl-1H-tetrazol-1-yl)-1H-imidazo-4-carboxamide (MTIC), and 5(4)-aminoimidazole-4(5)-carboxamide (AIC), and the metabolic pathway of TMZ.

Fig. 11.
IFO bioactivation pathway.

Fig. 12. Time-dependent response of IFO toxicity. IFO showed strong cytotoxicity to GBM cells after metabolism by hepatic cells. The cytotoxicity was significantly higher with CYP3A4 over-expressing C3A-sub28 cells than the parent C3A cells (*: $p < 0.05$).

Fig. 13. Dose-dependent response of IFO toxicity. IFO metabolized by C3A-sub28 cells showed the strongest cytotoxicity (*: $p < 0.05$). Without metabolism, the toxicity of IFO to GBM cells was considerably weaker ($p < 0.01$).

Fig. 14. Porous polymer scaffolds provide better media flow and media exchange with cellular aggregates.